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Review

A decade of progress in liver regenerative medicine

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ABSTRACT

Liver diseases can be caused by viral infection, metabolic disorder, alcohol consumption, carcinoma or injury, chronically progressing to end-stage liver disease or rapidly resulting in acute liver failure. In either situation, liver transplantation is most often sought for life saving, which is, however, significantly limited by severe shortage of organ donors. Until now, tremendous multi-disciplinary efforts have been dedicated to liver regenerative medicine, aiming at providing transplantable cells, microtissues, or bio-engineered whole liver via tissue engineering, or maintaining partial liver functions via extracorporeal support. In both directions, new compatible biomaterials, stem cell sources, and bioengineering approaches have fast-forwarded liver regenerative medicine towards potential clinical applications. Another important progress in this field is the development of liver-on-a-chip technologies, which enable tissue engineering, disease modeling, and drug testing under biomimetic extracellular conditions. In this review, we aim to highlight the last decade's progress in liver regenerative medicine from liver tissue engineering, bioartificial liver devices (BAL), to liver-on-a-chip platforms, and then to present challenges ahead for further advancement.

1. Introduction

The liver, as a vital organ, plays an essential role in protein synthesis and xenobiotic metabolism. Although the liver has a high degree of regenerative capacity, drugs, toxins, viral infections, cancer, or injury can still result in permanent tissue damage and liver function impairment, which will eventually lead to end-stage liver disease or rapidly cause acute liver failure [1]. To save patients' lives, liver transplantation is often conducted for long-term therapeutic efficacy [2], which is, however, considerably limited by the lack of immunologically compatible donor organs [3]. In addition, short-term and long-term immunosuppression needs to be administrated and maintained in patients, which inevitably deteriorates patient's health conditions and increases healthcare cost [4]. Compared to liver transplantation, hepatocyte transplantation, which is less invasive and can be performed repeatedly, has been used to treat acute liver failure [5]. However, hepatocyte transplantation suffers from a low degree of engraftment (less than 30%), and this method cannot restore long-term liver functions [5]. Thus, liver tissue engineering approaches have been extensively explored to provide transplantable microtissues or whole bioengineered liver for long-term hepatic functional restoration.

Until now, liver tissue engineering has significantly progressed in engineering cells, biomaterials and tissue architectures to fabricate transplantable liver microtissues [6–9] or bioengineered

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whole liver [10,11] over the last decade. Cell sources range from primary hepatocytes to induced pluripotent stem cells (iPSCs) derived hepatocyte-like cells, which are amenable to cell cultivation at a large scale and offer immunological compatibility for allogeneic transplantation. Biomaterial wise, hydrogel, gelatin methacryloyl (GelMA), and polyethylene glycol (PEG) have been evaluated in construction of 3D extracellular matrix (ECM), which is essential to provide spatial architectures and mechanical cues for stem cell differentiation and hepatocyte maturation. More importantly, perfusion-based culture systems have been introduced into 3D tissue culture, as opposed to traditional static 2D cell culture, to mimic extracellular environment *in vivo* for efficient mass exchange and cellular communication, especially in long-term culture [12]. It has been demonstrated that liver microtissues generated from stem cells with fine adjustment of biophysical and soluble factors exhibit enhanced liver functions in pigs [3] and mice [13,14]. Besides liver microtissues, bioengineered whole liver has also been clinically attractive, and pioneered research in this field has shown that decellularized whole liver can be reseeded with functional hepatocytes to rescue animals with induced liver failure [10,11].

Apart from liver tissue engineering, other liver regenerative medicine-based strategies such as bioartifical liver (BAL) devices [15] and liver-on-a-chip platforms [16,17] have been developed to mainly provide extracorporeal support [18] and pharmacological testing [19,20], respectively. Based on blood dialysis to remove metabolic wastes, BAL is incorporated with functional hepatocytes to partially restore a patient's liver functions while waiting for liver transplantation [18]. It has been clinically proven that temporal extracorporeal support offered by BAL for patients can provide beneficial effects on treating patients with liver diseases in clinical trials as previously reviewed [18]. In contrast to bulky BAL devices, the liver-on-a-chip platform is a miniaturized microscale device integrated with the capacity to precisely control temporal and spatial distribution of nutrients and growth factors, as well as delivery of physiological stimuli to cells under perfusion-based culture conditions [19,20]. Depending on the type of cells, scaffolds, growth factors, and biomechanical stimuli, the liver-on-a-chip platform can be adapted for versatile applications such as disease modeling, stem cell differentiation, drug screening, toxicity testing, and so on. Clearly, these two types of devices have significantly impacted the clinical and basic research related to liver regenerative medicine.

In this review, we therefore aim to summarize the progress of liver regenerative medicine including both regenerating transplantable liver microtissues and creating *in vitro* liver models for regeneration purposes from the last decade. In detail, we will focus on liver regenerative medicine including basic and translational research in liver tissue engineering, bioartificial liver devices (BAL) for extracorporeal support in clinical practice, and liver-on-a-chip platforms for drug testing (Fig. 1). We will first present the advances in four key components, namely cells, scaffolds, soluble factors and biophysical cues for liver tissue engineering [8,21,22], and their applications in generating transplantable liver microtissues or bioengineered whole liver. We will then further discuss the advances and applications of BAL and liver-on-a-chip technologies in the domain of liver regenerative medicine, benefiting from latest development of dynamic 3D cell culture systems. Finally, we envision the challenges ahead and research endeavor needed for further advancing liver regenerative medicine.

2. Liver tissue engineering

In general, two highly interacting components, namely cells and extracellular environment [23–27], are considerably investigated in tissue engineering. Cells including mature cells, progenitor cells or stem cells can be modulated to provide physiological functions as their counterparts *in vivo*. On the other hand, extracellular environment can be designed to recapitulate the hierarchy milieu of native tissues with consideration of biomechanical, biochemical and biophysical cues [28–32]. We, hereby, present the latest advances in liver tissue engineering with respect to cells, scaffolds, soluble factors, and biophysical factors.

2.1. Essential components for liver tissue engineering

2.1.1. Cells

Nowadays, primary hepatocytes remain the first choice for liver tissue engineering, because freshly isolated hepatocytes still maintain drug-metabolizing capacity *in vivo*. However, primary hepatocytes, when cultured *in vitro*, gradually lose their morphology and liver-specific functions such as carbohydrate metabolism, protein synthesis and cytochrome P450 activity. To overcome these drawbacks, a great deal of research effort has been focused on enhancing hepatocyte functions via developing 2D matrix [33], 3D scaffolds [34,35] and perfusion-based microfluidic systems [36]. For instance, to maintain key specific functions of primary hepatocytes, a monolayer coating of collagen was applied in a 2D culture system [33]. The hepatocytes, cultured on 2D collagen coating, maintained a typical honeycomb morphology and showed augmented gene expression (*e.g.,* vimentin, Zinc finger E-box-binding homeobox 1 (ZEB1) and snail-1). Further, primary hepatocytes, seeded on 3D scaffolds such as hydrogels [34] and polymer scaffolds [36] showed enhanced hepatocyte aggregation, spreading, and metabolic functions [34]. In a 3D perfusion-based microfluidic system, which constantly removes metabolic wastes and replenishes nutrients, cell morphology, cell viability and cell-cell fusion were significantly enhanced even when primary human hepatocytes were cultured in the absence of biochemical matrices for two weeks [37]. In another study, it was found that the dynamic flow maintained and stabilized the hepatocyte function through collagen secretion [38]. Despite the advances in culturing conditions, the use of primary hepatocytes still faces considerable technical obstacles in liver tissue engineering, since they need to be freshly isolated from patients for immune compatibility, and they tend to lose replication capacity over time *in vitro*.

To generate an unlimited supply of functional hepatocytes, a variety of stem cells, including embryonic stem cells (ESCs) [39], mesenchymal stem cells (MSCs) [40] and iPSCs [7], have been investigated. ESCs are derived from the inner cell mass of blastocysts, and they have self-renewal capability and pluripotency to differentiate into almost all cell types. A number of studies have shown that ESCs can be induced to differentiate into hepatocytes by chemical stimulation [41,42]. For example, ESCs differentiated into hepatoblasts when cultured in basal media supplemented with biochemical factors such as insulin and sodium selenite in a stepwise manner at sequential differentiation stages [41]. The derived hepatoblasts expressed its characteristic markers and liver-specific transcriptional factors such as AFP/hepatocyte nuclear factor 4α (HNF4α), EpCAM/HNF4α, and pan-cytokeratin/forkhead box protein A2 (FoxA2) during prolonged culture, indicative of the establishment of partial liver functions. In another study, cells differentiated from human ESCs, owing to enhanced interactions among cells in a 3D culture system, exhibited morphological and ultrastructural characteristics of primary hepatocytes under SEM and TEM [42]. In addition, enhanced and prolonged expression of liver-specific proteins such as albumin (ALB), phosphoenolpyruvate carboxykinase, and asialoglycoprotein receptor 1 was observed in the 3D aggregates [42]. These studies indicate the feasibility to differentiate hepatocytes from ESCs.

MSCs isolated from bone marrow [3,43,44], adipose tissue [29],